

WE CLAIM:

1. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:
 - 5 (a) culturing the mammalian cell in the presence and absence of the compound;
 - (b) assaying expression of at least one cellular gene in Table 2A in said cell in the presence of the compound with expression of said gene in the cell in the absence of the compound; and
 - 10 (c) identifying compounds that induce senescence when expression of at least one cellular gene in Table 2A is higher in the presence of the compound than in the absence of the compound.
2. A method according to claim 1, wherein the mammalian cell is a p53
15 deficient cell.
3. A method according to claim 1, wherein the mammalian cell is a tumor cell.
- 20 4. The method of claim 1, where expression of the cellular gene of Table 2A is detected by hybridization to a complementary nucleic acid.
5. The method of claim 1, wherein expression of the cellular gene of Table 2A is detected using an immunological reagent.
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6. The method of claim 1, wherein expression of the cellular gene of Table 2A is detected by assaying for an activity of the cellular gene product.
7. The method of claim 1, wherein the cellular gene is BTG1, BTG2, EPLIN,
30 WIP1, Maspin, MIC-1, IGFBP-6 or amphiregulin.

8. A method according to claim 1, wherein induction of at least one of the cellular genes in Table 2A is assayed using a recombinant mammalian cell comprising a reporter gene operably linked to a promoter from a cellular gene in Table 2A and detecting increased expression of the reporter gene in the presence of the compound than
5 in the absence of the compound.

9. A method according to claim 1, further comprising the steps of:
d) assaying expression of one or more genes in Table 2B; and
e) identifying compounds wherein expression of the genes in Table
10 2B is not greater in the presence of the compound than in the absence of the compound.

10. The method of claim 9, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.
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11. The method of claim 9, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

12. The method of claim 9, wherein expression of the cellular gene of Table
20 2B is detected by assaying for an activity of the cellular gene product.

13. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

- 25 (a) culturing the mammalian cell in the presence and absence of the compound;
- (b) assaying expression of at least one cellular gene in Table 2A in said cell in the presence of the compound with expression of said gene in the cell in the absence of the compound;
- 30 (c) assaying the recombinant mammalian cell for cell growth and morphological features of senescence; and
- (d) identifying compounds that induce senescence when expression of at least one cellular gene in Table 2A is higher in the presence of the compound

than in the absence of the compound and the cells are growth-inhibited and express morphological features of senescence in the presence of the compound.

5 14. A method according to claim 13, wherein the mammalian cell is a p53 deficient cell.

 15. A method according to claim 13, wherein the mammalian cell is a tumor cell.

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 16. The method of claim 13, where expression of the cellular gene of Table 2A is detected by hybridization to a complementary nucleic acid.

 17. The method of claim 13, wherein expression of the cellular gene of Table
15 2A is detected using an immunological reagent.

 18. The method of claim 13, wherein expression of the cellular gene of Table 2A is detected by assaying for an activity of the cellular gene product.

20 19. The method of claim 13, wherein the cellular gene is BTG1, BTG2, EPLIN, WIP1, Maspin, MIC-1, IGFBP-6 or amphiregulin.

 20. A method according to claim 13, wherein induction of at least one of the cellular genes in Table 2A is assayed using a recombinant mammalian cell comprising a
25 reporter gene operably linked to a promoter from a cellular gene in Table 2A and detecting increased expression of the reporter gene in the presence of the compound than in the absence of the compound.

 21. A method according to claim 13 further comprising the steps of:
30 e) assaying expression of one or more genes in Table 2B; and

f) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

5 22. A method according to claim 20 further comprising the steps of:

f) assaying expression of one or more genes in Table 2B; and

f) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

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23. The method of claims 21 or 22, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

15 24. The method of claims 21 or 22, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

25. The method of claims 21 or 22, wherein expression of the cellular gene of Table 2B is detected by assaying for an activity of the cellular gene product.

20 26. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

(a) producing a recombinant mammalian cell by introducing into said mammalian cell a recombinant expression construct comprising a promoter from a cellular gene in Table 2A operably linked to a reporter gene;

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(b) culturing the recombinant mammalian cell in the presence and absence of the compound;

(c) assaying expression of the reporter gene in said recombinant cell in the presence of the compound with expression of said reporter gene in the recombinant cell in the absence of the compound; and

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- (d) identifying compounds that induce senescence when gene expression of the reporter gene is higher in the presence of the compound than in the absence of the compound.

5 27. A method according to claim 26, wherein the mammalian cell is a p53 deficient cell.

28. A method according to claim 26, wherein the mammalian cell is a tumor cell.

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29. The method of claim 26, wherein the promoter of the cellular gene is a promoter from BTG1, BTG2, EPLIN, WIP1, Maspin, MIC-1, IGFBP-6 or amphiregulin.

15 30. A method according to claim 26, further comprising the steps of:
e) assaying expression of one or more genes in Table 2B; and
f) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

20 31. The method of claim 30, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

32. The method of claim 30, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

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33. The method of claim 30, wherein expression of the cellular gene of Table 2B is detected by assaying for an activity of the cellular gene product.

30 34. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

- (a) producing a recombinant mammalian cell by introducing into said mammalian cell a recombinant expression construct comprising a

promoter from a cellular gene in Table 2A operably linked to a reporter gene;

(b) culturing the recombinant mammalian cell in the presence and absence of the compound;

5 (c) assaying expression of the reporter gene in said recombinant cell in the presence of the compound with expression of said reporter gene in the recombinant cell in the absence of the compound;

(d) assaying the recombinant mammalian cell for cell growth and morphological features of senescence; and

10 (e) identifying compounds that induce senescence when reporter gene expression is higher in the presence of the compound than in the absence of the compound and the cells are growth-inhibited and express morphological features of senescence in the presence of the compound.

15 35. A method according to claim 34, wherein the mammalian cell is a p53 deficient cell.

36. A method according to claim 34, wherein the mammalian cell is a tumor cell.

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37. The method of claim 34, wherein the promoter of the cellular gene is a promoter from a BTG1, BTG2, EPLIN, WIP1, Maspin, MIC-1, IGFBP-6 or amphiregulin.

25 38. A method according to claim 34, further comprising the steps of:
f) assaying expression of one or more genes in Table 2B; and
g) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

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39. The method of claim 38, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

40. The method of claim 38, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

5 41. The method of claim 38, wherein expression of the cellular gene of Table 2B is detected by assaying for an activity of the cellular gene product.

42. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

- 10 (a) culturing the mammalian cell in the presence and absence of the compound;
- (b) assaying expression of at least one cellular gene in Table 1 in said cell in the presence of the compound with expression of said gene in the cell in the absence of the compound; and
- 15 (c) identifying compounds that induce senescence when expression of at least one cellular gene in Table 1 is lower in the presence of the compound than in the absence of the compound.

20 43. A method according to claim 42, wherein the mammalian cell is a p53 deficient cell.

44. A method according to claim 42, wherein the mammalian cell is a tumor cell.

25 45. The method of claim 42, where expression of the cellular gene of Table 1 is detected by hybridization to a complementary nucleic acid.

46. The method of claim 42, wherein expression of the cellular gene of Table 1 is detected using an immunological reagent.

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47. The method of claim 42, wherein expression of the cellular gene of Table 1 is detected by assaying for an activity of the cellular gene product.

48. The method of claim 42, wherein the cellular gene is HFH-11, STEAP, RHAMM, INSIG1, LRPR1.

5 49. A method according to claim 42, wherein inhibition of at least one of the cellular genes in Table 1 is assayed using a recombinant mammalian cell comprising a reporter gene operably linked to a promoter from a cellular gene in Table 1 and detecting decreased expression of the reporter gene in the presence of the compound than in the absence of the compound.

10 50. A method according to claim 41, further comprising the steps of:
d) assaying expression of one or more genes in Table 2B; and
e) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of
15 the compound.

51. A method according to claim 48, further comprising the steps of:
d) assaying expression of one or more genes in Table 2B; and
e) identifying compounds wherein expression of the genes in Table
20 2B is not greater in the presence of the compound than in the absence of the compound.

52. The method of claims 50 or 51, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

25 53. The method of claims 50 or 51, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

54. The method of claims 50 or 51, wherein expression of the cellular gene of
30 Table 2B is detected by assaying for an activity of the cellular gene product.

55. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

- (a) culturing the mammalian cell in the presence and absence of the compound;
- 5 (b) assaying expression of at least one cellular gene in Table 1 in said cell in the presence of the compound with expression of said gene in the cell in the absence of the compound;
- (c) assaying the recombinant mammalian cell for cell growth and morphological features of senescence; and
- 10 (d) identifying compounds that induce senescence when expression of at least one cellular gene in Table 1 is lower in the presence of the compound than in the absence of the compound and the cells are growth-inhibited and express morphological features of senescence in the presence of the compound.

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56. A method according to claim 55, wherein the mammalian cell is a p53 deficient cell.

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57. A method according to claim 55, wherein the mammalian cell is a tumor cell.

58. The method of claim 55, where expression of the cellular gene of Table 1 is detected by hybridization to a complementary nucleic acid.

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59. The method of claim 55, wherein expression of the cellular gene of Table 1 is detected using an immunological reagent.

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60. The method of claim 55, wherein expression of the cellular gene of Table 1 is detected by assaying for an activity of the cellular gene product.

61. The method of claim 55, wherein the cellular gene is HFH-11, STEAP, RHAMM, INSIG1, LRPR1.

62. A method according to claim 55, wherein inhibition of at least one of the cellular genes in Table 1 is assayed using a recombinant mammalian cell comprising a reporter gene operably linked to a promoter from a cellular gene in Table 1 and detecting
5 decreased expression of the reporter gene in the presence of the compound than in the absence of the compound.

63. A method according to claim 55, further comprising the steps of:

e) assaying expression of one or more genes in Table 2B; and

f) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

64. A method according to claim 62, further comprising the steps of:

f) assaying expression of one or more genes in Table 2B; and

f) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

65. The method of claims 63 or 64, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

66. The method of claims 63 or 64, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

67. The method of claims 63 or 64, wherein expression of the cellular gene of Table 2B is detected by assaying for an activity of the cellular gene product.

68. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

(a) producing a recombinant mammalian cell by introducing into said mammalian cell a recombinant expression construct comprising a

promoter from a cellular gene in Table 1 operably linked to a reporter gene;

(b) culturing the recombinant mammalian cell in the presence and absence of the compound;

5 (c) assaying expression of the reporter gene in said recombinant cell in the presence of the compound with expression of said reporter gene in the recombinant cell in the absence of the compound; and

(d) identifying compounds that induce senescence when expression of the reporter gene is lower in the presence of the compound than in the absence
10 of the compound.

69. A method according to claim 68, wherein the mammalian cell is a p53 deficient cell.

15 70. A method according to claim 68, wherein the mammalian cell is a tumor cell.

71. The method of claim 68, wherein the promoter of the cellular gene is a promoter from HFH-11, STEAP, RHAMM, INSIG1, LRPR1.

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72. A method according to claim 68, further comprising the steps of:

e) assaying expression of one or more genes Table 2B; and

f) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of
25 the compound.

73. The method of claim 72, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

30 74. The method of claim 72, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

75. The method of claim 72, wherein expression of the cellular gene of Table 2B is detected by assaying for an activity of the cellular gene product.

76. A method for identifying a compound that induces senescence in a mammalian cell, the method comprising the steps of:

- (a) producing a recombinant mammalian cell by introducing into said mammalian cell a recombinant expression construct comprising a promoter from a cellular gene in Table 1 operably linked to a reporter gene;
- (b) culturing the recombinant mammalian cell in the presence and absence of the compound;
- (c) assaying expression of the reporter gene in said recombinant cell in the presence of the compound with expression of said reporter gene in the recombinant cell in the absence of the compound;
- (d) assaying the recombinant mammalian cell for cell growth and morphological features of senescence; and
- (e) identifying compounds that induce senescence when reporter gene expression is lower in the presence of the compound than in the absence of the compound and the cells are growth-inhibited and express morphological features of senescence in the presence of the compound.

77. A method according to claim 76, wherein the mammalian cell is a p53 deficient cell.

78. A method according to claim 76, wherein the mammalian cell is a tumor cell.

79. The method of claim 76, wherein the promoter of the cellular gene is a promoter from HFH-11, STEAP, RHAMM, INSIG1, LRPR1.

80. A method according to claim 76, further comprising the steps of:
g) assaying expression of one or more genes in Table 2B; and

g) identifying compounds wherein expression of the genes in Table 2B is not greater in the presence of the compound than in the absence of the compound.

5 81. The method of claim 80, where expression of the cellular gene of Table 2B is detected by hybridization to a complementary nucleic acid.

 82. The method of claim 80, wherein expression of the cellular gene of Table 2B is detected using an immunological reagent.

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 83. The method of claim 80, wherein expression of the cellular gene of Table 2B is detected by assaying for an activity of the cellular gene product.

 84. A compound that induces senescence in a mammalian cell wherein the
15 compound is identified according to a method of claim 9, 21, 22, 30, 38, 50, 51, 63, 64, 72 or 80.

 85. A compound according to claim 84 that is a non-retinoid compound.

20 86. A method for assessing efficacy of a treatment of a disease or condition relating to abnormal cell proliferation or neoplastic cell growth, the method comprising the steps of:

 (a) obtaining a biological sample comprising cells from an animal having a
25 disease or condition relating to abnormal cell proliferation or neoplastic cell growth before treatment and after treatment;

 (b) comparing expression of at least one gene in Table 1, 2A or 2B after treatment with expression of said genes before treatment; and

 (c) determining that said treatment has efficacy for treating the disease or
30 condition relating to abnormal cell proliferation or neoplastic cell growth if expression of at least one gene in Table 2A and 2B is higher after treatment than before treatment or expression of at least one gene in Table 1 is lower after treatment than before treatment.

87. The method of Claim 86, wherein the biological sample comprises tumor cells.

5 88. The method of Claim 86, wherein the gene is a cellular gene in Table 2A.

89. The method of claim 88, wherein at least one cellular gene is BTG1, BTG2, EPLIN, WIP1, Maspin, MIC-1, IGFBP-6 or amphiregulin.

10 90. The method of Claim 86, wherein the gene is a cellular gene in Table 1.

91. The method of claim 90, wherein the cellular gene is HFH-11, STEAP, RHAMM, INSIG1, LRPR1.

15 92. The method of claim 86, where expression of the cellular gene of Tables 1, 2A or 2B is detected by hybridization to a complementary nucleic acid.

93. The method of claim 86, wherein expression of the cellular gene of Tables 1, 2A or 2B is detected using an immunological reagent.

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94. The method of claim 86, wherein expression of the cellular gene of Tables 1, 2A or 2B is detected by assaying for an activity of the cellular gene product.

25 95. A method for treating a disease or condition relating to abnormal cell proliferation or neoplastic cell growth, the method comprising the steps of administering to an animal having said disease or condition a therapeutically effective amount of a compound produced according to the method of claims 9, 21, 22, 30, 38, 50, 51, 63, 64, 72 or 80 that induces senescence in abnormally proliferating or neoplastic cells.

30 96. The method of claim 95 wherein the compound is a non-retinoid compound.

97. A method for identifying a compound that inhibits senescence-associated induction of cellular gene expression, the method comprising the steps of:

- (a) contacting the cell with a cytotoxic agent at a concentration of said agent that inhibits cell growth;
- 5 (b) assaying the cell in the presence and absence of the compound for changes in expression of cellular genes induced when cells become senescent; and
- (c) identifying the compound as an inhibitor of senescence-associated induction of cellular gene expression if expression of the cellular genes of subpart (b) is induced in the absence of the compound but is not induced in
10 the presence of the compound.

98. The method of claim 97, wherein the cellular gene is cyclin D1, serum-inducible kinase, CYR61, prosaposin, transforming growth factor α (TGF α), kallikrein 7, calpain-L2, neurosin, plasminogen activator urokinase, amyloid beta (A4) precursor
15 protein (β APP), or integral membrane protein 2B (BRI/ITM2B).

99. The method of claim 97, where expression of the cellular gene is detected by hybridization to a complementary nucleic acid.

20 100. The method of claim 97, wherein expression of the cellular gene is detected using an immunological reagent.

101. The method of claim 97, wherein expression of the cellular gene is detected by assaying for an activity of the cellular gene product.
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102. A method according to claim 97, wherein the mammalian cell is a p53 deficient cell.

103. A method according to claim 97, wherein the mammalian cell is a tumor
30 cell.

104. A method for identifying a compound that inhibits senescence-associated induction of cellular gene expression, the method comprising the steps of:

- 5 (a) producing a recombinant mammalian cell by introducing into said mammalian cell a recombinant expression construct comprising a promoter from cyclin D1, serum-inducible kinase, CYR61, prosaposin, transforming growth factor α (TGF α), kallikrein 7, calpain-L2, neurosin, plasminogen activator urokinase, amyloid beta (A4) precursor protein (β APP), or integral membrane protein 2B (BRI/ITM2B) operably linked to a reporter gene;
- 10 (b) contacting the cell with a cytotoxic agent at a concentration of said agent that inhibits cell growth;
- (c) assaying expression of the reporter gene in said recombinant cell in the presence of the compound with expression of said reporter gene in the recombinant cell in the absence of the compound
- 15 (d) identifying the compound as an inhibitor of senescence-associated induction of cellular gene expression if expression of the cellular genes of subpart (c) is induced in the absence of the compound but is not induced in the presence of the compound.

20 105. The method of claim 104, where expression of the cellular gene is detected by hybridization to a complementary nucleic acid.

25 106. The method of claim 104, wherein expression of the cellular gene is detected using an immunological reagent.

107. The method of claim 104, wherein expression of the cellular gene is detected by assaying for an activity of the cellular gene product.

30 108. A method for determining treatment efficacy in an animal treated with a compound that induces cellular senescence, the method comprising the steps of:

- (a) assaying a biological fluid from the animal before and after treatment for a senescence marker; and
- (b) determining that the treatment is effective when the amount of the marker detected after treatment is greater than the amount of the marker detected before treatment.

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109. The method of claim 108, wherein the senescence marker is maspin, MIC-1, IGFBP-6, or amphiregulin.

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110. The method of claim 108, wherein the bodily fluid is blood, urine, effusions, ascitic fluid, saliva, cerebrospinal fluid, cervical secretions, vaginal secretions, endometrial secretions, gastrointestinal secretions, bronchial secretions, sputum, or secretions or washings from the breast.

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111. The method of claim 108, where the senescence marker is detected by hybridization to a complementary nucleic acid, using an immunological reagent or by assaying for an activity of the cellular gene product.

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